the mitochondrial fraction. However, extensive washing reduced the total activity of the mitochondrial fraction by 75% indicating that the activity in this fraction may have been due to insufficient washing in the first 3 experiments. The activity of the microsomal fraction was also reduced by washing.

Summary. The data indicate that the *de novo* methionine methyl group synthesizing system resides to a large extent in the liver of the rat. Within the liver cells more than 75% of the synthesizing ability was shown to be in the supernatant fraction when the particulate fractions were well washed to remove any adhering cell sap.

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Infectious and Oncogenic Effect of DNA Extracted from Cells Infected with Polyoma Virus. (29124)

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DNA extracted from cultured mouse cells infected with polyoma virus is infectious *in vitro*. We have previously reported that when this DNA is injected into hamsters it causes the appearance of circulating specific antibodies and tumors(1); the purpose of the present study was to determine with greater accuracy certain conditions affecting manifestation of these specific biological properties.

Material and methods. For the viral DNA extraction, use was made of embryonic mouse cells in tissue cultures collected 4 to 6 days after infection with polyoma virus and of uninfected control cultures. These cells were cultured in a medium containing casein hydrolysate with 10% inactivated horse serum. Maintenance medium contained only 2% serum. The polyoma virus strain used came from the laboratory of R. Dulbecco, and was placed at our disposal by K. Habel. For each experiment we used material from 15 to 20 one-liter Roux bottle cultures.

The cells were extracted with phenol, either without prior treatment or after heating to temperatures of 80 to 100°C for periods of 15 to 45 minutes. Details of the phenol treatment have been described(2). Final extracts were centrifuged for 1 hour at 130,000 *g*, and resuspended in 0.5 M NaCl to contain 400 to 600 μg of DNA per ml. They were then injected subcutaneously (0.15 ml) or intracerebrally (0.03 ml) into either newborn golden hamsters, or animals 40 days of age. Other animals were injected with DNA preparations treated with deoxyribonuclease (DNase) for 1 hour at 37°C. Certain animals were given a 6% solution of para-aminosalicylic acid (PAS), since in certain experiments this had been used as the aqueous phase in the extraction of the DNA. Finally, some animals were not injected but left in contact with hamsters inoculated with DNA. The infectious and oncogenic properties of the original virus strain were determined by subcutaneous inoculation of va-

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riable quantities of the virus into newborn hamsters. All animals were bled on the 30th day and sera were tested for specific anti-polyoma virus antibodies by the hemagglutination-inhibition (HI) reaction with guinea pig erythrocytes in the presence of 8 hemagglutinating units of virus. Most animals dying during the experimental period (182-400 days) were autopsied and their organs examined histologically for presence of tumors.

An *in vitro* determination of the infectious activity of DNA preparations was carried out in most of the experiments. Primary cultures of kidney cells from 10-day-old mice in tubes were inoculated with 0.5-1 ml of DNA in 0.5 M NaCl (*i.e.*, approximately 50 μg), and the tubes were kept at 37°C for 20 minutes. Cultures were then washed 2 × with medium, medium added, and incubated at 37°C. In parallel experiments, cells were inoculated with DNA hydrolyzed by DNase or with 0.5 M NaCl in phosphate buffer, to check for the possible appearance of a latent virus in the cultures. The cytopathogenic effect (CPE) was observed directly or after fixation and staining of the cultures with haemalun-eosin or by the fluorescent antibody technique.

Results. A summary of the results of hamster inoculation is given in Table I. A total of 35 newborn animals were given DNA. On the 30th day, 34 were still alive and all had HI antibodies, with titers varying between 1,920 and 30,200. Of these 34 hamsters, 6 could not be autopsied, 2 showed no lesions, and 26 had developed tumors after an interval of about 3 months.

The tumors were generally found in the subcutaneous tissue, but sometimes were present in the lungs and liver in association with hemorrhagic disease. Three cases of intrathoracic localization were observed. Histological examination revealed the presence of fibro-sarcomatous and angio-sarcomatous lesions, typical of polyoma tumors.

Of 35 newborn hamsters given DNA from non-infected cells, either in its untreated state or treated with DNase, 22 were still alive on the 350th day, none developed tumors or HI antibodies (not shown in Table I). A similar negative result was obtained in 26 hamsters that had been inoculated either with DNA extracted from infected cells and hydrolyzed with DNase, or with 6% PAS. Of 12 untreated animals kept in contact with hamsters that had been given infectious DNA, a single animal developed a subcutaneous tumor and antibodies.

The inoculated adult hamsters also reacted to administration of DNA irrespective of route of administration, but it appeared that, dose for dose, they were less sensitive than newborn hamsters, the incidence of tumors being less (5 out of 17) and the interval before their appearance being longer (5 to 6 months).

DNA viral preparations proved to be infectious for mouse kidney cultures (Table II). The typical CPE appeared after the 8th day and was not seen in cultures inoculated with DNA which had been treated with DNase or with DNA extracted from uninfected control cells. The supernatant medium from these negative cultures was harvested on the 12th day of incubation and given one blind passage in fresh cultures again with negative results. Heating of the cells before DNA extraction, different aqueous phase or temperature during the extraction process did not appear to affect significantly the efficiency of extraction.

Discussion. In the experiments reported here it has been demonstrated that DNA extracted from cells infected with polyoma virus is capable of producing virus infection and tumors in the hamster in parallel with the previously demonstrated(2,3,4,5) infectivity for cells in tissue culture. That the positive results were due to DNA rather than residual intact virus was established by the negative results both *in vivo* and in tissue culture when the extracts were treated with DNase. The finding of others(6) which we have confirmed (unpublished results) that no infectious DNA can be obtained from hamster cells transformed by polyoma virus but now free of demonstrable virus, suggests that the infectious DNA extracted in these experiments is of viral origin rather than cellular. That the appearance of infection and

TABLE I. Effects of Administration to Hamsters of DNA from Cultures of Mouse Embryos Infected with Polyoma Virus.

Exp	Treatment	No.	Age (days)	Presence of HI antibodies at 30 days	Animals examined p.m.		Avg interval to appearance of tumors (days)	Localization of tumors			
					With tumor	Without tumor		S.C.	Liver	Lung	Intrathoracic fibrosarcomata
I	DNA sc .15 ml	11	3	11	11	—	98	8	4	3	—
	Not inoculated, in contact with former	8	3	1	1	7	151	1	—	—	—
II	DNA sc .15 ml or .10 ml*	7	2–3	7	6	1	108	4	3	3	1
	DNA + DNase sc .15 ml or .10 ml*	7	2–3	—	—	2	—	—	—	—	—
	6% sol of PAS sc .15 ml or .10 ml*	7	2–3	—	—	1	—	—	—	—	—
III	DNA sc .15 ml	5	2–4	5	2	—	77	2	—	—	1
	Not inoculated, in contact with former	4	2–4	—	—	—	—	—	—	—	—
	DNA + DNase sc .15 ml	6	2–4	—	—	2	—	—	—	—	—
IV	DNA sc .15 ml	11	2–4	11	9	1	97	9	1	—	1
	DNA + DNase sc .15 ml	6	2–4	—	—	4	—	—	—	—	—
V	DNA sc .15 ml	10	40	6	3	5	158	3	—	—	—
	DNA IC .04 ml	7	40	5	2	5	164	—	1	—	1
	6% sol PAS .15 ml	7	40	—	—	5	—	—	—	—	—

* These animals were given 2 injections of DNA with an interval of 24 hr.

TABLE II. Experimental Conditions for Extraction of DNA and *in vitro* Test of Its Infectious Properties.

	Heating of homogenized cells before extraction		Conditions of extraction			CPE in tissue cultures			
							Time of appearanse (days) No. pos/No. inoc		
Exp	Infected	Normal	Nature of aqueous phase	Time of action of phenol* (min.)	Temp °C	Duration of observation (days)	DNA	DNA + DNase	NaCl .5 M
I	None		1†	5,3,3	50	45	(8–30) 5/6	0/6	0/6
II	15 min. 100°C		2‡	15,10,10	20	45	(18) 1/6	0/6	0/6
III	45 min. 80°C		2	6,3,3	50	35	(9–19) 3/6	0/6	0/6
IV	45 min. 80°C		2	6,3,3	50	—	—	—	—
V	45 min. 75°C		2	15,10,10	20	45	(8–11) 6/7	0/6	0/6
VI		45 min. 80°C	2	6,3,3	50	35	0/6	0/6	0/6
VII		45 min. 80°C	2	6,3,3	50	—	—	—	—

* Phenol saturated by a .02 M phosphate buffer—5×10^{-4} M EDTA.
† Phase 1: .02 M phosphate buffer—5×10^{-4} M EDTA—pH 7.3.
‡ Phase 2: the cells were heated in 5 to 10 ml of 5×10^{-4} M EDTA, .14 M NaCl, .05 M Na-citrate then 6_p 100 (wt/vol) of p. amino-salicylic acid was added.

tumors observed following inoculation of the hamsters with viral DNA was not due to a latent polyoma infection in the hamsters is unlikely since all hamsters in our colony are negative for polyoma antibodies and there was no evidence of infection or tumors in animals inoculated with PAS or DNA from normal cells. The possibility that the majority of positive hamsters were infected by contact with a few original reactors is not likely since neither the mothers nor the uninoculated control animals, with one exception, developed antiviral antibodies or tumors during the long period of observation. Furthermore, it has been shown that excretion of polyoma virus by infected hamsters is quantitatively low in amount(7).

For comparative purposes, Table III shows the appearance of polyoma antibodies and tumors when a range of infectious doses of our stock virus is inoculated into newborn hamsters. On the basis of average time before appearance of tumors, their frequency and localization, it would appear that our DNA preparations had biological activity equivalent to approximately 50-500 $TCID_{50}$ of infectious virus. However, the amount of DNA inoculated per hamster was the extract from approximately 5×10^7 $TCID_{50}$ of virus. The tremendous drop in infectivity of the extracted DNA may be due to a number of factors: (a) less efficient adsorption and penetration by DNA as compared to whole virus; (b) dilution of viral DNA with heterologous cellular DNA, as shown for transforming bacterial DNA(8); (c) enzymic degradation of the DNA by blood and cellular DNases; (d) physico-chemical modification of the DNA during the extraction, however the polyoma virus DNA(9), as well as papilloma virus DNA(10) is resistant to treatments which usually denature these macromolecules.

On the basis of the present experiments it cannot be determined whether the tumors produced in animals inoculated with viral DNA preparations were induced by the DNA itself or by virus produced in subsequent cycles of multiplication occurring in the animal. That such virus multiplication took place is apparent from the antiviral antibody response. It is interesting that infectious vi-

TABLE III. Titration in Newborn Hamsters of Stock Polyoma Virus Used in Present Experiments.

Dose inoculated $TCID_{50}$ (.1 ml sc)	No. of animals inoculated	Presence of HI antibodies on 30th day	Avg interval to appearance of tumors (days)	Animals examined		Localization of tumors				
				With tumors	Without tumors	S.C.	Liver	Lung	Heart	Kidney	Misc
.1	7	1	68	1	5	1	–	–	–	–	–
1	10	6	106	4	5	4	1	–	–	–	–
5	8	8	90	4	3	4	–	–	1	–	1*
50	13	13	75	11	1	7	2	1	1	–	–
500	12	12	72	12	–	9	3	3	–	–	–
5,000,000	8		29	8	–	1	8	4	3	4	–
Control culture medium from uninoculated mouse embryo cultures	10	0	–	–	9	–	–	–	–	–	–

* Intra-abdominal fibrosarcoma.

rus could not be demonstrated in the induced tumors by inoculating tumor extracts into tissue culture, by direct culture of tumor cells or by production of antibodies on tumor transplantation in the hamster. However, this is also true of many tumors produced by inoculation of newborn hamsters with whole infectious virus.

Latarjet *et al*(11) and Hays and Carr(12) have obtained tumors of the polyoma type on mouse inoculation with nucleic acid preparations from mouse leukemic tissues or parotid tumors. It is likely that their results were due to extracted polyoma DNA or to the presence of polyoma virus which resisted the nucleic acid extraction procedure.

The results reported by Harris and Chesterman(13) in experiments similar to ours were of questionable significance since only 3 of 52 newborn hamsters inoculated with polyoma DNA developed tumors. Furthermore, they did not treat the DNA with DNase as control *in vivo* and their nucleic acid preparations lost their infectivity *in vitro* in the presence of antiviral antibody.

Summary. Thirty-five newborn hamsters were injected subcutaneously (approx. 70 μg) or intracerebrally (15 μg) with DNA from polyoma-infected cultures of embryonal mouse cells. All animals showed circulating antibodies and 26 of them presented tumors, mostly of subcutaneous localization, after intervals of 2 to 3 months. Fully grown hamsters appeared to be less sensitive; of 17 animals, 11 developed antibodies and 5 developed tumors. The same DNA preparations were shown to be capable of infecting primary cultures of mouse kidney cells. All the control series, in which use was made of DNA treated with DNase or of DNA originating from non-infected cells, have given negative results both *in vivo* and *in vitro*.

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Effect of Experimental Allergic Encephalomyelitis on Guinea Pig Plasma Lipid Fractions.* (29125)

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Kies *et al*(1) noted the occurrence of visible lipemia in sera of severely paralyzed guinea pigs after the induction of experimental allergic encephalomyelitis (EAE). This lipemia included markedly elevated triglyceride and less marked increases of cholesterol and phospholipid. There was also a significant increase in the lipid-staining material which migrated electrophoretically with albumin.

Dole(2) and Gordon and Cherkes(3) described the small but metabolically active free fatty acid (FFA) fraction of plasma which has subsequently been recognized to have a major role in fat metabolism(4). Goodman(5) noted that free fatty acids in the plasma are bound to albumin, and described the albumin binding sites.

On the hypothesis that the lipemia of EAE might be brought about by increased mobilization of FFA from fat depots with its subsequent reappearance as triglyceride, phospholipid, and cholesterol esters, plasma FFA concentrations were measured during early and late stages of EAE. Just as had been observed with total plasma lipids, plasma FFA values were highest in severely affected animals, but even at 15 or 16 days after injection, when many of the animals had not yet shown definite neurological signs, the FFA values were significantly higher than normal.

Since paralyzed animals do not eat and since fasting can increase FFA(2,3,6), the effect of fasting was compared to the effect of EAE on blood lipid fractions. Although fasting may increase all lipid fractions, certain quantitative differences were noted between fasting and EAE.

Experimental. Animals: Disease-free mixed color guinea pigs bred in a closed colony in the Animal Production Section at the National Institutes of Health were used for all of the experiments described. The animals were young males varying in weight from 250-500 g depending upon the individual experiment. All animals were housed in large general purpose cages, 6 to a cage, on a bed of cedar shavings. They were obtained from the stock colony one week prior to injection and given a regular stock diet for guinea pigs (NIH formula A) plus a daily supplement of greens (lettuce and kale).

Induction of experimental allergic enceph-

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